CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

081701

The AOAC Research Institute hereby certifies the method known as:

Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay

manufactured by

Oxoid Ltd part of Thermo Fisher Scientific
Wade Rd
Basingstoke, Hampshire
RG24 8PW, UK

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date October 11, 2024
Expiration Date December 31, 2025

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METHOD NAME
Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay

CATALOG NUMBERS
A56846, A56847

ORIGINAL CERTIFICATION DATE
August 18, 2017

PRINCIPLE OF THE METHOD

Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay enables real-time PCR detection and differentiation of *Salmonella* species, *Salmonella* ser. Typhimurium, and *Salmonella* Enteritidis from meat, production environment samples, and primary production samples. The assay is based on TaqMan™ real-time PCR technology. Dye-labeled probes target unique DNA sequences specific to *Salmonella* ser. Typhimurium, *Salmonella* ser. Enteritidis and all *Salmonella* species, and an internal positive control. Target DNA, if present, is amplified by PCR and detected in real-time using fluorescence hydrolysis probe chemistry. The fluorescence signal that is generated is detected by the real-time PCR instrument and interpreted by the analysis software. The assay includes an internal positive control for each reaction to confirm that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

CERTIFIED CLAIM STATEMENT: The Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR method is certified for the detection of *Salmonella* Typhimurium and *Salmonella* Enteritidis within the scope of Tables 1 and 2 and with modifications indicated in Table 3.

***Certification includes**:*

1. Applied Biosystems™ QuantStudio™ 5 Real-Time PCR with Thermo Scientific™ RapidFinder™ Analysis software version 3.0 or later.
2. Applied Biosystems™ 7500 Fast Real-Time PCR with Applied Biosystems™ RapidFinder™ Express software version 2.0 or later.
3. Alternative confirmation procedure: Direct streak from the primary enrichment onto Thermo Scientific *Brilliance* Salmonella Agar. The O group status of presumptive *S*. Enteritidis or *S*. Typhimurium results were confirmed using the agar slope confirmation procedure detailed in the RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay User Guide. Presumptive Salmonella species results were confirmed using the Oxoid Salmonella latex kit.

Table 1. Method Performance Claims

			Enrichment Conditions				Reference	
Matrix[a]	Target	Test Portion	Broth[a]	Volume	Temperature	Time	Method[b]	Claim[c]
Raw chicken thighs with skin	*Salmonella* sp.	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	NSDD
	S. Enteritidis	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	SDD-C
Raw chicken wings with skin	*S*. Typhimurium	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	NSDD
Chicken nuggets	*Salmonella* sp.	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	NSDD
	S. Typhimurium	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	NSDD
Raw pork sausage	*Salmonella* sp.	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	NSDD
	S. Typhimurium	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	NSDD
	S. Enteritidis	25 g	BPW+n	225 mL	41.5 ± 1°C	14-18 h	MLG 4.09	SDD-C
Stainless steel (4”x4”)	*Salmonella* sp.	Sponge	BPW+n	225 mL	41.5 ± 1°C	14-18 h	BAM Ch. 5	NSDD

	S. Typhimurium	Sponge	BPW+n	225 mL	41.5 ± 1°C	14-18 h	BAM Ch. 5	NSDD
Raw ground turkey	*Salmonella* sp.	375 g	pwBPW+n	1125 mL	41.5 ± 1°C	20-24 h	MLG 4.09	SDD-C[d]
	S. Typhimurium	375 g	pwBPW+n	1125 mL	41.5 ± 1°C	20-24 h	MLG 4.09	NSDD
Chicken carcass rinse	*Salmonella* sp.	30 mL	pwBPW+n	90 mL	41.5 ± 1°C	16-20 h	MLG 4.09	NSDD
	S. Enteritidis	30 mL	pwBPW+n	90 mL	41.5 ± 1°C	16-20 h	MLG 4.09	NSDD
	S. Typhimurium	30 mL	pwBPW+n	90 mL	41.5 ± 1°C	16-20 h	MLG 4.09	SDD-C
Shell eggs	*Salmonella* sp.	20 eggs	pwBPW+n	2 L	37 ± 1°C	16-20 h	BAM Ch. 5	SDD-C
		1 mL	BPW	10 mL	37 ± 1°C	4 h		
	S. Enteritidis	20 eggs	pwBPW+n	2 L	37 ± 1°C	16-20 h	BAM Ch. 5	NSDD
		1 mL	BPW	10 mL	37 ± 1°C	4 h		

[a] BPW(ISO)+n = Buffered Peptone Water (ISO formulation) with 12 mg/L novobiocin, BPW= Buffered Peptone Water (ISO formulation).

[b] MLG = USDA FSIS Microbiology Laboratory Guidebook, BAM = Bacteriological Analysis Manual.

[c] NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence. Expert opinion is that the method is appropriate for its intended use. SDD-C = Statistical difference detected with a positive bias for the Candidate method.
Matrix – Matrix inoculated with organism(s) listed to the right.

[d] NSDD in the Independent Laboratory Study.

Table 2. Method Selectivity

	Enrichment		Inclusivity Strains		Exclusivity Strains	
Target	Broth[a]	Temp., °C	No. Tested	No. Positive	No. Tested	No. Positive
Salmonella sp.	BPW+n	41.5 ± 1°C	200[b]	200	45[c]	0
Salmonella Enteritidis	BPW+n	41.5 ± 1°C	50	50		
Salmonella Typhimurium	BPW+n	41.5 ± 1°C	50	50		
Salmonella sp.	BPW+n	41.5 ± 1°C	196[d,e]	195[f]	45[c,d]	3 (7500 Fast) / 2 (QS5)[g]
Salmonella Enteritidis	BPW+n	41.5 ± 1°C	50[d]	48[f]		
Salmonella Typhimurium	BPW+n	41.5 ± 1°C	50[d]	50		

[a] BPW(ISO)+n = Buffered Peptone Water (ISO formulation) with 12 mg/L novobiocin.

[b] Comprising 100 strains *Salmonella* species including 2 strains *Salmonella* enterica subsp. *bongori*, 5 strains *Salmonella* enterica subsp. *arizonae*, 5 strains *S. enterica* subsp. *diarizonae*, 5 strains *S. enterica* subsp. *indica*, 5 strains *S. enterica* subsp. *houtenae*, 1 strain *S. enterica* subsp. *salamae*, and the remaining strains represent 76 serovars *S. enterica* subsp. *enterica*), 50 strains of *Salmonella* Enteritidis, and 50 strains of *Salmonella* Typhimurium.

[c] Comprising 35 species.

[d] Testing performed using the Thermo Scientific™ KingFisher™ Flex™ Purification System.

[e] Comprising 96 strains *Salmonella* species (including 2 strains *Salmonella* enterica subsp. *bongori*, 5 strains *Salmonella* enterica subsp. *arizonae*, 5 strains *S. enterica* subsp. *diarizonae*, 5 strains *S. enterica* subsp. *indica*, 5 strains *S. enterica* subsp. *houtenae*, 5 strains *S. enterica* subsp. *salamae*, and the remaining strains represent 68 serovars *S. enterica* subsp. *enterica*.); 50 strains of *Salmonella* Enteritidis, 50 strains of *Salmonella* Typhimurium.

[f] One *Salmonella* Enteritidis isolate was positive the *Salmonella* sp. and negative for *Salmonella* Enteritidis with the Applied Biosystems 7500 Fast PCR Instrument and the Applied Biosystems QuantStudio 5 PCR Instrument. Another *Salmonella* Enteritidis isolate was negative for *Salmonella* sp.

and *Salmonella* Enteritidis with the Applied Biosystems QuantStudio 5 PCR Instrument. Both isolates were found to have an O:9 antigen mutation causing the cells to not bind properly to the Dynabeads during sample lysis, resulting in a false negative result.

[g] Three species (*Citrobacter freundii* and two *Serratia marcescens*) originally detected with the Applied Biosystems 7500 Fast Real Time PCR Instrument and two species (*Escherichia blattae* and *Proteus vulgaris*) originally detected with the Applied Biosystems QuantStudio 5 Real Time PCR Instrument. When retested after enrichment in the candidate method enrichment broth (BPW (ISO) with 12 mg/mL novobiocin), all were negative by both instruments.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	August 2017	Original certification includes raw chicken thighs with skin, raw chicken wings with skin, chicken nuggets, raw pork sausage, stainless steel (4”x4”, sponge).	Certification Report
2	May 2018	Level 2 Modification: Addition of raw ground turkey, chicken carcass rinse, shell eggs.	Modification Report 1
3	October 2020	Level 2 Modification: Evaluation to upgrade software for Thermo Scientific RapidFinder Analysis (RFA) PCR software to version 1.1 for the Applied Biosystems QuantStudio 5 Real-Time PCR.	Modification Report 2
4	October 2020	Level 2 Modification: Evaluation to upgrade the software for the Applied Biosystems RapidFinder Express (RFE) to version 2.0 designed for use with the Applied Biosystems 7500 Fast Real-Time PCR.	Modification Report 3
5	July 2022	Level 2 Modification: Changes made to improve handling steps and visual indicators.	Modification Report 4
6	January 2024	Level 2 Modification: Addition of automated lysis procedure and PCR setup procedure.	Modification Report 5
7	October 2024	Level 1 Modification: Method name change from Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit; and Thermo Scientific Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit to Thermo Scientific SureTec Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay.	NA[a]

[a] Not Applicable